

Static Stretching Combined with Conscious Slower Breathing May Increase Parasympathetic Activity and Reduce Stress in Adult Women

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Abstract

Background: Women are thought to be more susceptible to stress than men in a stressful society, and reducing stress is crucial for women to maintain their health. Static stretching (SST) is applied in various fields to not only increase muscle flexibility but also reduce stress. Additionally, conscious slower breathing (CSB) predominates parasympathetic activity, causing a relaxing effect. These results indicate that combining SST and CSB may be more useful in reducing stress. However, to the best of our knowledge, the effect of this combination remains unclear. Objective: This study aimed to elucidate the effects of the combination of SST and CSB on autonomic activity and stress in adult women. Methods: Eleven healthy Japanese adult female participants performed SST with nonconscious natural breathing for 20 min. The same participants performed SST in combination with CSB (2 s inspiratory and 4 s expiratory) for 20 min on another day. Salivary cortisol and chromogranin A levels were measured before and after stretching as stress markers of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system. The coefficient of variation of the R-R interval (CVR-R) and high-frequency component (HF), which reflect parasympathetic nerve activity, and heart rate and low-frequency component (LF)/HF ratio, which reflect sympathetic nerve activity, were measured before, during, and after stretching. Results: SST decreased cortisol levels but with no significant changes in chromogranin A, heart rate, CVR-R, HF, or LF/HF ratio. The combination of SST and CSB increased CVR-R and HF levels in addition to decreasing cortisol levels but with no significant changes in chromogranin A, heart rate, or LF/HF levels. **Conclusion:** These results indicate that the combination of SST and CSB may increase parasympathetic activity and reduce stress. However, future randomized controlled trials with larger sample sizes should support this conclusion.

Keywords

Static Stretching, Conscious Slower Breathing, Autonomic Activity, Heart Rate Variability, Stress

1. Introduction

Women, in the modern stressful society, are thought to be more susceptible to stress than men [1] [2] [3]. This is because women are more susceptible to hormonal imbalances than men due to menstruation, pregnancy, childbirth, and menopause [4] [5]. Excessive stress harms daily health by causing physical and mental symptoms such as insomnia, depression, anxiety, and headaches [6]. Therefore, relieving daily stress is crucial for women to live a healthy life.

Static stretching (SST) and deep breathing are frequently introduced as simple ways to relieve stress and relax at home or work [7] [8] [9].

SST is a calisthenic exercise that slowly stretches a muscle and maintains its stretched state without giving any recoil or momentum. It relieves muscle tension by stretching the muscles, increasing flexibility, and making the parasympathetic nervous system dominant [8]. Yoga stretching is a modified exercise to be comfortable and easy for beginners, holding relaxed poses with natural breathing, and is very similar to SST. It reduces salivary cortisol levels and increases parasympathetic activity [7] [10]. These results indicate that SST is effective in reducing stress and improving relaxation. However, we frequently hold our breath while stretching our muscles. Breath-holding causes muscle stiffness and increased blood pressure, making full relaxation impossible. Therefore, breathing by performing SST without holding the breath is important to achieve a relaxing effect during SST.

Breathing comprises exhalation and inspiration, which are controlled by the sympathetic and parasympathetic nerves, respectively [11]. In contrast, inspiration and expiration influence sympathetic and parasympathetic nerve activity, respectively. In particular, conscious breathing indicates that controlling the balance between sympathetic and parasympathetic nerve activity is possible [12] [13]. This helps alleviate various physical and psychological symptoms such as dysautonomia, anxiety, and depression [14]. We recently demonstrated that conscious slower breathing (CSB: 2 s inhalation + 4 s exhalation/one breath) predominates parasympathetic activity, causing a relaxing effect [15]. These results indicate that combining SST and CSB may be more useful in reducing stress and enhancing relaxation. However, to the best of our knowledge, the antistress and

relaxation effects of this combination remain unclear.

Stress response involves two major central mediators: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) [1]. The hypothalamus is the first brain region to respond to stressors, which promotes the activity of the HPA axis and SNS. Hence, HPA axis activation releases glucocorticoid cortisol from adrenocortical cells. SNS activation releases noradrenaline from peripheral nerve endings and adrenaline from the adrenal medulla. Increased SNS activity combined with decreased parasympathetic activity causes autonomic imbalance. Therefore, stress intensity can be biochemically assessed by measuring cortisol, which reflects HPA axis activity, and catecholamines, which reflect SNS activity [16].

Saliva is frequently used to measure stress-related substances because sample collection is quick, easy, and noninvasive [17]. However, unlike cortisol, cate-cholamine levels are generally low and are rapidly degraded, making their concentrations in saliva difficult to measure. Chromogranin A is an acidic glyco-protein that is present in the adrenal medulla and sympathetic nerve endings and is coreleased with catecholamines. It is used as a surrogate marker for cate-cholamines because it can be stable and easily measured [17] [18].

Heart rate-related information, such as heart rate, coefficient of variation of R-R interval (CVR-R), and power spectral analysis of heart rate variability (HRV), is frequently used clinically to determine the balance of autonomic nervous activity [19]. Heart rate and CVR-R are thought to better reflect sympathetic and parasympathetic functions, respectively [20]. The high-frequency component (HF, 0.15 - 0.4 Hz) of the HRV power spectrum reflects parasympathetic activity, and the low-frequency component (LF, 0.04 - 0.15 Hz)/HF ratio reflects the dominance of sympathetic activity over autonomic activity [15] [21].

This study aimed to elucidate the effects of SST combined with CSB on autonomic nervous activity and stress in healthy Japanese adult women. Participants performed SST for 20 min with and without CSB (2 s inhalation + 4 s exhalation). Salivary stress markers (cortisol and chromogranin A) and heart rate-related information (heart rate, CVR-R, HF component, and LF/HF ratio) were measured as objective evaluation markers. The effectiveness of combining SST with CSB was investigated by comparing them to SST with natural breathing (i.e., without conscious breathing).

2. Methods

2.1. Subjects

This study included 11 healthy Japanese adult women aged 24 - 40 years (mean age: 32.2 ± 5.1 years, height: 161.0 ± 6.4 cm, weight: 48.4 ± 4.4 kg, and body mass index: 18.7 ± 1.9) who provided consent to the purpose of this study. Exclusion criteria for participant recruitment were those who were unsure of their physical fitness level, those with pre-existing medical conditions, and those tak-

ing medication. Participants were instructed not to consume caffeine on the day of the study, not to eat 1 h before the experiment, and to avoid strenuous exercise, get enough sleep, and refrain from drinking alcohol the day before the study. Their compliance with these instructions was confirmed through selfreports and interviews on the day of the study.

2.2. Ethics

The Ethics Committee of Juntendo University Graduate School of Health and Sports Science, Japan (Grad. 29 - 59) approved the study protocol which complies with the Helsinki Declaration. All subjects provided informed consent.

2.3. Experimental Procedures and Analytical Methods

The participants (N = 11) performed SST with nonconscious natural breathing. On another day (approximately 2 weeks later), they (N = 11) underwent SST combined with CSB (2-s inspiratory and 4-s expiratory). Figure 1 shows the experimental procedure. The time required for the experiment was 90 min. All subjects rested for 15 min in the sitting position, followed by 15 min in the supine position. After 25 min of practice, they then performed SST or SST combined with CSB for 20 min, followed by a 15 min rest in the supine position. The intervention was conducted in a quiet room with room temperature set at 25°C to 28°C and humidity 40% to 60%. A professional instructor taught how to perform SST and CSB in a 25 min practice session. In addition, stretching and breathing exercises were performed under the explicit instructions and demonstrations of the instructor during the 20 min intervention.

A wearable biometric information tracer, M-BIT (Institute of Man and Science, Inc., Tokyo, Japan), was attached to the left chest of patients for 90 min to acquire heart rate-related data, such as heart rate, CVR-R, HF component, and LF/HF ratio, according to a previously reported procedure [15]. We used the data obtained during a 5-min rest before practice, 20 min stretching, and 15 min rest after stretching as data before, during, and after stretching, respectively.



Figure 1. Experimental schedule. All subjects rested for 15 min in the sitting position, followed by 15 min in the supine position. After 25 min of practice, they then performed SST or SST combined with CSB for 20 min, followed by a 15 min rest in the supine position. An M-BIT to obtain HRV data was attached to the patient's left chest for 90 min. Pre-, during-, and poststretch data were obtained during a 5 min rest before practice, a 20 min SST or SST + CSB, and a 15 min rest after stretching. Saliva samples for measuring cortisol and chromogranin A levels were collected before and after stretching.

Saliva samples for measuring cortisol and chromogranin A levels were collected using Saliva Collection Aid (Salimetrics LLC, USA) before the practice and after SST or SST + CSB. Salivary concentrations of cortisol and chromogranin A were measured using enzyme-linked immunosorbent assay. Saliva sample collection and component analysis were performed according to our previously established protocol [17].

2.4. Static Stretching

This study used SST consisting of 13 types of stretches: 1) gluteus maximus stretch with knees facing forward, 2) hamstring stretch, 3) adductor magnus stretch, 4) gracilis stretch, 5) gluteus maximus stretch with knees outward, 6) rectus femoris stretch, 7) vastus medialis stretch, 8) vastus lateralis stretch, 9) supine hip circle, 10) supine arm circles, 11) torso twist stretch, 12) ankle flex-ion-extension stretch, and 13) single arm and leg elongation stretch. All of these stretches were performed in the supine position, as shown in Figure 2(A) (gluteus maximus stretch) and Figure 2(B) (vastus lateralis muscle stretch). Participants performed sequentially from stretches 1 to 13. Each stretch was performed in three sets on each side, left and right. One set consisted of a 10 s stretching followed by a 2 s rest.

2.5. Statistical Analyses

Data are represented as the mean ± standard error of the mean (SEM). All



Figure 2. Stretching performed in the supine position. (A) A gluteus maximus stretch; (B) A vastus lateralis muscle stretch.

measured parameters were calculated as percentage changes relative to the prestretch values. Dunnett's multiple comparison test or Mann-Whitney U test was used to determine the statistical significance of the relative change data after performing a two-way repeated measures analysis of variance (ANOVA) for the group factor (SST and SST + CSB), time factor (pre, during and poststretching), and interaction of both factors. *P*-values of <0.05 indicated significance. Graph-Pad Prism version 8.4.2 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis.

3. Results

3.1. Cortisol

Figure 3 shows the effects of SST and SST + CSB on salivary cortisol levels. The relative rate of change to the prestretch value was calculated to compare the changes between the two groups (**Figure 3(A)**), and **Figure 3(B)** shows the results. Factorial analysis revealed a significant effect for the time factor ($F_{1,20} = 7.254$, P < 0.05). However, no significant differences were found for the group factor ($F_{1,20} = 0.023$, P = 0.882) or interaction between the factors of time and group ($F_{1,20} = 0.076$, P = 0.786).

Post hoc analyses revealed that cortisol levels were significantly decreased compared with prestretch values in both the SST (P < 0.01) and SST + CSB (P < 0.05) groups, although these changes were not significantly different between the groups.



Figure 3. Effects of SST and SST + CSB on salivary cortisol levels. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in cortisol, calculated as a percentage of the prestretch value, and (B) shows the results. The relative values were statistically analyzed using a two-way repeated measures ANOVA followed by post hoc analysis, Mann-Whitney U test. **P* < 0.05 and ***P* < 0.01 vs pre-stretching value. No significant differences were observed between the two groups.



(A) Actual measurement values and relative change rate of salivary chromogranin A

Figure 4. Effects of SST and SST + CSB on salivary chromogranin A (CgA) concentrations. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in CgA calculated as a percentage of the prestretch values, and (B) shows the results. The relative values were statistically analyzed using two-way repeated measures ANOVA. No significant differences were observed for the time factor, group factor, or their interaction.

3.2. Chromogranin A

Figure 4 shows the effects of SST and SST + CSB on salivary chromogranin concentrations. The relativechange to the prestretch value was calculated for each group (**Figure 4(A)**) and **Figure 4(B**) shows the results. The factorial analysis revealed no significant effects for the time factor ($F_{1,20} = 3.783$, P = 0.066), group factor ($F_{1,20} = 0.002$, P = 0.967), or their interaction ($F_{1,20} = 0.004$, P = 0.953). These results indicated that both the SST and SST + CSB groups demonstrated no significant effect on chromogranin A.

3.3. Heart Rate

Figure 5 shows the changes in heart rate before, during, and after stretching in the SST and SST + CSB groups. The relative change to the prestretch value was calculated for each group (**Figure 5(A)**), and **Figure 5(B)** shows the results. The factorial analysis revealed a significant effect for the time factor ($F_{2,40} = 15.81$, *P* < 0.001) but not for the group factor ($F_{1,20} = 0.003$, *P* = 0.960) and their interaction ($F_{2,40} = 0.4110$, *P* = 0.666).

Post hoc analysis revealed that heart rate during stretching was significantly increased compared with prestretch values in both the SST (P < 0.01) and SST + CSB (P < 0.05) groups, although these were small increases within the range of 5% - 6% (approximately 3 - 4 beats/min) compared with prestretch values. After stretching, both returned to their prestretch values. These changes over time were not significantly different between the two groups.



(A) Actual measurement values and relative change rate of mean heart rate

Figure 5. Effects of SST and SST + CSB on heart rate. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in heart rate calculated as a percentage of the prestretch values, and (B) shows the results. The relative values were statistically analyzed using a two-way repeated measures ANOVA followed by post hoc analysis. **P* < 0.05 and ***P* < 0.01 vs prestretch value (Dunnett'smultiple comparison test). No significant differences were observed between the two groups (Mann-Whitney U test).

3.4. CVR-R

Figure 6 shows the effects of SST and SST + CSB on CVR-R. The relative change to the prestretch value was calculated for each group (**Figure 6(A)**), and **Figure 6(B)** shows the results. The factorial analysis revealed significance for the time factor ($F_{2,40} = 8.101$, P < 0.01) and interaction between factors of time and group ($F_{2,40} = 7.609$, P < 0.01). No significant differences were found for the group factor ($F_{1,20} = 3.453$, P = 0.078).

Post hoc analyses revealed no significant differences in the CVR-R values during and after stretching compared with the prestretch values in the SST group. In contrast, the CVR-R value during stretching in the SST + CSB group increased significantly compared with the prestretch value (P < 0.01) and returned to the prestretch value after stretching. The CVR-R value during stretching in the SST + CSB group increased significantly compared with that in the SST group (P < 0.01) but with no significant difference between the two groups after stretching.

3.5. HF Component

Figure 7 shows the effects of SST and SST+CSB on the HF component. The relative change to the prestretch value was calculated for each group (**Figure 7(A)**), and **Figure 7(B)** shows the results. The factorial analysis revealed a significant effect for the interaction between the factors of time and group ($F_{2, 40} = 7.885$, P < 0.01). No significant effects were found for the time factor ($F_{2, 40} = 1.527$, P = 0.230) and group factor ($F_{1, 20} = 3.595$, P = 0.073).

	Actual m	neasurement v	alue (%)	Relative change rate (%)					
Group	Pre	During	Post	Pre	During	Post			
SST	6.21 ± 0.53	6.27 ± 0.29	6.39 ± 0.42	100 ± 8.5	101 ± 4.8	103 ± 6.8			
SST + CSB	5.12 ± 0.44	7.11 ± 0.47	5.88 ± 0.30	100 ± 8.6	$139\pm9.3^{**,\dagger\dagger}$	115 ± 5.9			
(B) Relative CVR-R change									
lative changes in CVR-R (%		ł	**	-Ö	SST + C	CSB ∳			
Re	-	pre	(during	p	ost			

(A) Actual measurement values and relative change rate of CVR-R

Figure 6. Effects of SST and SST + CSB on CVR-R. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in CVR-R calculated as a percentage of the prestretch values, and (B) shows the results. The relative values were statistically analyzed using a two-way repeated measures ANOVA followed by post hoc analysis. "*P* < 0.01 vs prestretch value (Dunnett'smultiple comparison test) and "†*P* < 0.01 vs SST group (Mann-Whitney U test).

Post hoc analyses revealed no significant differences in the HF values during and after stretching compared with the prestretch values in the SST group. In contrast, the HF value during stretching in the SST + CSB group significantly increased compared with the prestretch value (P < 0.01), and returned to the prestretch value after stretching. The HF value during stretching in the SST + CSB group significantly increased compared with that in the SST group (P < 0.05) but with no significant difference between the two groups after stretching.

3.6. LF/HF Ratio

Figure 8 shows the effects of SST and SST + CSB on the LF/HF ratio. The relative change rate to the prestretch value was calculated (**Figure 8(A)**), and **Figure 8(B)** shows the results. The factorial analysis revealed no significant effects for the time factor ($F_{2,40} = 0.268$, P = 0.766), group factor ($F_{1,20} = 2.900$, P = 0.104), or their interaction ($F_{2,40} = 1.534$, P = 0.228). These results indicated that both the SST and SST + CSB groups had no significant effect on the LF/HF ratio.

4. Discussion

Stretching is mainly classified into two types: dynamic and static stretching [22] [23]. Dynamic stretching is a flexibility exercise that moves the body rhythmically. Physiologically, it increases sympathetic activity, heart rate, blood flow,

and body temperature, causing easier force application and smoother movement. Therefore, it is considered suitable for warming up before athletic competitions and for injury prevention [24]. In contrast, static stretching, which is the subject of this study, is a flexibility exercise that slowly stretches muscles while in a stationary state. Unlike dynamic stretching, it increases parasympathetic activity and decreases sympathetic activity, heart rate, blood flow, and body temperature [25] [26]. Therefore, it is thought to have calming and relaxing effect [8] [10]. Performing mild to moderate SST before sleep increases parasympathetic nerve activity and improves sleep quality, including sleep onset [27] [28]. Conversely, a study investigating HR and HRV before, during, and after stretching exercises in subjects with low flexibility levels revealed that SST increases sympathetic activity and decreases parasympathetic activity [29].

The present study revealed that SST increased heart rate by 5% - 6% (approximately 3 - 4 beats/min) compared with prestretch values of 60 - 70 beats/min (Figure 5). This increase demonstrated a statistically significant difference, but it is considered a negligible variation within the normal range when compared with heart rates of approximately 100 beats/min during dynamic stretching and walking exercise [25]. Therefore, our results indicate that the stretching exercise used in this study did not reduce or affect sympathetic activity. Additionally, the absence of significant changes in chromogranin A (Figure 4) and LF/HF ratio (Figure 8), which were measured as indicators of sympathetic activity, support this finding [17] [18] [21]. SST also did not significantly change CVR-R and HF (Figure 6 and Figure 7), which were measured as indicators of parasympathetic activity [20] [21]. However, SST significantly reduced cortisol (Figure 3), a stress hormone mediated through the HPA axis [1], which is consistent with previous studies [7] [10]. Altogether, these results indicate that the SST applied in this study reduces stress but does not affect autonomic activity. Our results differed from previous studies that reported that SST increased parasympathetic activity and decreased sympathetic activity [8] [10] [25] [26]. A possible reason for this difference may be the different protocols used for the SST tasks. In particular, Sakai et al. evaluated the effects of a 10 min SST of the triceps surface muscles through motor control [8], whereas we evaluated the effects of a 20 min SST primarily targeting the gluteal, hamstrings, and quadriceps muscles in 13 different poses (Figure 2). These results indicate that the effects may vary depending on the type of static stretching and the duration of exercise.

As described above, SST decreased cortisol levels but with no significant changes in chromogranin A, heart rate, CVR-R, HF, or LF/HF ratio. Conversely, SST combinedwith CSB increased CVR-R and HF levels (**Figure 6** and **Figure 7**) in addition to decreasing cortisol levels (**Figure 3**) but with no significant changes in chromogranin A, heart rate, and LF/HF levels (**Figure 4**, **Figure 5** and **Figure 8**). The reduction in cortisol level was not significantly different between the SST and SST + CSB groups (**Figure 3**), indicating that the reduced effect was due to SST rather than CSB. On the contrary, the increase in CVR-R and HF values was not observed with SST but was newly observed with SST + CSB (**Figure 6** and

0	Actual measurement value (ms ²)			Relative change rate (%)						
Group	Pre	During	Post	Pre	During	Post				
SST	10.78 ± 1.53	7.91 ± 0.97	11.13 ± 1.50	100 ± 14.2	73 ± 9.0	103 ± 13.9				
SST + CSB	7.95 ± 1.28	13.79 ± 2.47	8.17 ± 0.67	100 ± 16.1	174 \pm 31.0 **,†	102 ± 8.5				
(B) Relative HF change										
elative changes in HF (%) 001 002 002 002 002 002 002 002 002 002	- - -		**		ST+CSB)				
Ř		pre	du	ring	pos	st				

(A) Actual measurement values and relative change rate of HF component

Figure 7. Effects of SST and SST + CSB on the HF component. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in the HF component calculated as a percentage of the prestretch values, and (B) shows the results The relative values were statistically analyzed using a two-way repeated measures ANOVA followed by post hoc analysis. "*P* < 0.01 vs prestretch value (Dunnett's multiple comparison test) and [†]*P* < 0.05 vs SST group (Mann-Whitney U test).



Figure 8. Effects of SST and SST + CSB on the LF/HF ratio. Data represent the mean \pm SEM (n = 11). (A) shows the relative changes in the LF/HF ratio calculated as a percentage of the prestretch values, and (B) shows the results. The relative values were statistically analyzed using two-way ANOVA. No significant differences were observed for the time factor, group factor, or their interaction.

Figure 7), indicating that the increase in these parasympathetic activity markers was due to CSB rather than SST. SST with or without CSB did not affect chromogranin A, heart rate, and LF/HF levels (**Figure 4**, **Figure 5** and **Figure 8**), re-

vealing that CSB, similar to SST, has little effect on sympathetic activity. Taken together with the result that CSB increases parasympathetic activity, this reveals that CSB predominates parasympathetic activity in the autonomic nervous system. Our previous study reporting that CSB (2 s of inhalation, 4 s exhalation) used in this study predominated parasympathetic activity, resulting in a relaxing effect, in healthy adult women, supported this result [15]. Additionally, CSB has increased parasympathetic nervous activity and relaxed patients with emotional disorders such as premenstrual syndrome, stress, anxiety, and depression [14] [30]. Altogether, this study indicates that the combination of SST and CSB may be useful in reducing stress and inducing relaxation in women.

This study had several limitations. First, the sample size was small and the same participants performed both SST and SST + CSB in this study, thus our conclusion needs to be supported by future randomized controlled trials with larger sample sizes that include not only healthy subjects but also patients with psychological stress. Second, this study evaluated the effects of single stretching. Future studies are warranted to further clarify the usefulness of SST combined with CSB by evaluating its continuous or habitual effects. Finally, the type and duration of SST and its combination with CSB should be further investigated to provide a more effective combination of SST and CSB.

5. Conclusion

This study reveals that SST with CSB may increase parasympathetic activity and reduce stress. However, future randomized controlled trials with larger sample sizes should confirm this conclusion.

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Authors' Contributions

Mami Sakurai: Conceptualization, Data curation, Formal analysis, investigation, methodology, visualization, and writing-original draft preparation. Yasushi Ikarashi: Visualization and Writing: Review and Editing. Masahiro Tabuchi: Writing, review, and editing. Ailing Hu: Formal analysis and resources. Takuji Yamaguchi: Resources and Project administration. Hiroyuki Kobayashi: Supervision.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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